Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 251–255, January 1992
Biochemistry

Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney

(gene/G protein-coupled receptor/neuropeptide)

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Communicated by Roger Guillemin, October 3, 1991

ABSTRACT Somatostatin is a tetradecapeptide that is widely distributed in the body. It acts on multiple organs including brain, pituitary, gut, exocrine and endocrine pancreas, adrenals, thyroid, and kidneys to inhibit release of many hormones and other secretory proteins. In addition, it functions as a neuropeptide affecting the electrical activity of neurons. Somatostatin exerts its biological effects by binding to specific high-affinity receptors, which appear in many cases to be coupled to GTP-binding proteins. Here we report the cloning, functional expression, and tissue distribution of two different somatostatin receptors (SSTRs). SSTR1 and SSTR2 contain 391 and 369 amino acids, respectively, and are members of the superfamily of receptors having seven transmembrane segments. There is 46% identity and 70% similarity between the amino acid sequences of SSTR1 and SSTR2. Stably transfected Chinese hamster ovary cells expressing SSTR1 or SSTR2 exhibit specific somatostatin binding, with an apparently higher affinity for somatostatin-14 than somatostatin-28, an NH_2-terminally extended form of somatostatin-14. RNA blotting studies show that SSTR1 and SSTR2 are expressed at highest levels in jejunum and stomach and in cerebrum and kidney, respectively. A SSTR1 probe hybridized to multiple DNA fragments in *Eco*RI digests of human and mouse DNA, indicating that SSTR1 and SSTR2 are members of a larger family of somatostatin receptors. Thus, the biological effects of somatostatin are mediated by a family of receptors that are expressed in a tissue-specific manner.

Somatostatin is a tetradecapeptide that was first isolated from hypothalamic extracts and shown to be a potent inhibitor of growth hormone secretion from the anterior pituitary (1). Subsequent studies have shown that it is widely distributed occurring in the central nervous system and peripheral tissues such as stomach, intestine, and pancreas (2). Somatostatin has diverse physiological effects that are tissue-specific (2). It can function as a neurotransmitter as well as a hormone. Its hormonal effects include suppression of release of many pituitary, pancreatic, and gastrointestinal hormones and other secretory proteins.

Somatostatin-14 is a member of a family of somatostatin-like peptides that also includes an NH_2-terminally extended form, somatostatin-28 (3, 4). The two principal bioactive forms of somatostatin, somatostatin-14 and -28, are derived by tissue-specific proteolytic processing of prosomatostatin, the 92-amino acid precursor of somatostatin-14 and -28 (5) and are present at various concentrations in different tissues. Although somatostatin-14 and -28 may have common effects on target tissues, they show different potencies, suggesting that their actions are mediated by different receptors (2). For example, somatostatin-14 appears to be relatively more selective for inhibition of glucagon and gastric acid secretion, whereas somatostatin-28 is a more specific inhibitor of growth hormone, insulin, and pancreatic exocrine secretion (6).

Somatostatin-14 and -28 exert their biological effects by binding to high-affinity receptors that appear in many cases to be coupled to GTP-binding (G) proteins (7, 8). Pharmacological studies indicate that there are at least two subtypes of somatostatin receptor (SSTR) (9, 10), presumably reflecting receptors that are selective for either somatostatin-14 or -28. As part of a project characterizing the proteins expressed in human insulin-secreting pancreatic β-cells, especially those that may be involved in the regulation of insulin secretion such as the SSTR, we amplified mRNA sequences in human pancreatic islet RNA that could encode G protein-coupled receptors, using the polymerase chain reaction (PCR) (11). Here we report the cloning and sequence‖ of genes encoding two different SSTRs that have distinct tissue distributions and the functional characterization of SSTR1 and SSTR2. Thus, the diverse biological effects of somatostatin appear to be mediated by tissue-specific expression of a family of SSTRs.

MATERIALS AND METHODS

General Methods. Standard methods were carried out as described in Sambrook *et al.* (12) and as described (13). Human islets were provided by D. W. Scharp and P. E. Lacy (Washington University School of Medicine). RNA was isolated by using the guanidinium isothiocyanate/CsCl procedure. DNA sequencing was done by the dideoxynucleotide chain-termination procedure (14) after subcloning appropriate DNA fragments into M13 mp18 or mp19. Both strands were sequenced.

Cloning of cDNAs and Genes Encoding G Protein-Coupled Receptors Expressed in Human Islets. Ten micrograms of total human pancreatic islet RNA were reverse-transcribed by using an oligo(dT) primer and avian myeloblastosis virus reverse transcriptase (Molecular Genetic Resources, Tampa, FL). Sequences related to G protein-coupled receptors were amplified by using PCR and the degenerate oligonucleotide primers described in Libert *et al.* (11). The PCR products were separated on a 1% low-melting-temperature agarose gel, and DNA fragments between 400 and 500 base pairs (bp) were eluted from the agarose, ligated into M13 mp18, and sequenced. The PCR product encoding a G protein-coupled

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Abbreviations: SSTR, somatostatin receptor; G protein, GTP-binding protein.

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‖The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M81829–32).

receptor termed hGPR81 was ^{32}P-labeled by nick-translation and was used to screen a human genomic library (15) and to isolate the gene encoding this new member of the family of receptors having seven transmembrane segments. Standard hybridization conditions were used [16 hr at 42°C in 50% formamide/0.75 M NaCl/0.075 M sodium citrate/20 mM sodium phosphate buffer, pH 6.5/0.1% SDS/100 μg of sonicated and denatured salmon testes DNA per ml/10% dextran sulfate/0.04% polyvinylpyrrolidone/0.04% Ficoll/0.04% bovine serum albumin] (16), and filters were washed at 50°C in 0.015 M NaCl/0.0015 M sodium citrate, pH 7/0.1% SDS before autoradiography. Mouse SSTR1 and human and mouse SSTR2 genes were isolated from human and mouse genomic libraries (Stratagene) by hybridization as described (13).

Heterologous Expression of Human SSTR1 and Mouse SSTR2 in Chinese Hamster Ovary (CHO) Cells and Binding Experiments. A 1.5-kb *Pst* I/*Xmn* I fragment of the human SSTR1 gene and a 1.2-kb *Xba* I fragment of the mouse SSTR2 gene were inserted into the mammalian expression vectors pCMV6b and pCMV6c, respectively (gifts of B. Chapman, Chiron Corp.). The resulting constructs were cotransfected with pSV2neo into the dihydrofolate reductase-deficient CHO cell line DG44 by using Lipofectin reagent (GIBCO/BRL). Stable transfectants were selected and maintained in α minimal essential medium (GIBCO/BRL) containing 400 μg of G418 per ml. Each cell line was grown to confluency in six-well 35-mm diameter plates at 37°C. The cells were washed with buffer containing 10 mM Hepes, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 150 mM NaCl, and 1% (wt/vol) bovine serum albumin (all at pH 7.4) and were stabilized in the same buffer for 30 min at 22°C. Cells were incubated in duplicate with 1 ml of buffer containing bacitracin (1 mg/ml) and [^{125}I-Tyr^{11}]somatostatin-14 (50,000 cpm, Amersham) alone or with somatostatin-14 or somatostatin-28 (Bachem) at the indicated concentrations for 30 min at 22°C. Incubated cells were washed twice with 2-ml portions of ice-cold buffer and were dissolved in 1 ml of 8 M urea/3 M acetic acid. The solution of dissolved cells was transferred to a tube, the well was washed with 1 ml of the same solution, and the combined extracts were measured for radioactivity by use of a γ-counter.

RESULTS AND DISCUSSION

Cloning of SSTR Gene. mRNA sequences in human pancreatic islet RNA that encode G protein-coupled receptors

A

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                 1                                  10
                Met Phe Pro Asn Gly Thr Ala Ser Ser Pro Ser Ser Ser Pro Ser Pro
CCCCCTCAGCTGGG  ATG TTC CCC AAT GGC ACC GCC TCC TCT CCT TCC TCC TCT CCT AGC CCC

            20                  Ala Cys             30      Ser
Ser Pro Gly Ser Cys Gly Glu Gly Gly Gly Ser Arg Gly Pro Gly Ala Gly Ala Ala Asp
AGC CCG GGC AGC TGC GGC GAA GGC GGC GGC AGC AGG GGC CCC GGG GCC GGC GCT GCG GAC

            40                                      50
Gly Met Glu Glu Pro Gly Arg Asn Ala Ser Gln Asn Gly Thr Leu Ser Glu Gly Gln Gly
GGC ATG GAG GAG CCA GGG CGA AAT GCG TCC CAG AAC GGG ACC TTG AGC GAG GGC CAG GGC

            60                                      70
Ser Ala Ile Leu Ile Ser Phe Ile Tyr Ser Val Val Cys Leu Val Gly Leu Cys Gly Asn
AGC GCC ATC CTG ATC TCT TTC ATC TAC TCC GTG GTG TGC CTG GTG GGG CTG TGT GGG AAC

            80                                      90
Ser Met Val Ile Tyr Val Ile Leu Arg Tyr Ala Lys Met Lys Thr Ala Thr Asn Ile Tyr
TCT ATG GTC ATC TAC GTG ATC CTG CGC TAT GCC AAG ATG AAG ACG GCC ACC AAC ATC TAC

            100                                     110
Ile Leu Asn Leu Ala Ile Ala Asp Glu Leu Leu Met Leu Ser Val Pro Phe Leu Val Thr
ATC CTA AAT CTG GCC ATT GCT GAT GAG CTG CTC ATG CTC AGC GTG CCC TTC CTA GTC ACC

            120                                     130
Ser Thr Leu Leu Arg His Trp Pro Phe Gly Ala Leu Leu Cys Arg Leu Val Leu Ser Val
TCC ACG TTG TTG CGC CAC TGG CCC TTC GGT GCG CTG CTC TGC CGC CTC GTG CTC AGC GTG

            140                                     150
Asp Ala Val Asn Met Phe Thr Ser Ile Tyr Cys Leu Thr Val Leu Ser Val Asp Arg Tyr
GAC GCG GTC AAC ATG TTC ACC AGC ATC TAC TGT CTG ACT GTG CTC AGC GTG GAC CGC TAC

            160                                     170
Val Ala Val Val His Pro Ile Lys Ala Ala Arg Tyr Arg Arg Pro Thr Val Ala Lys Val
GTG GCC GTG GTG CAT CCC ATC AAG GCG GCC CGC TAC CGC CGG CCC ACC GTG GCC AAG GTA

            180                                     190
Val Asn Leu Gly Val Trp Val Leu Ser Leu Leu Val Ile Leu Pro Ile Val Val Phe Ser
GTA AAC CTG GGC GTG TGG GTG CTA TCG CTG CTC GTC ATC CTG CCC ATC GTG GTC TTC TCT

            200                                     210
Arg Thr Ala Ala Asn Ser Asp Gly Thr Val Ala Cys Asn Met Leu Met Pro Glu Pro Ala
CGC ACC GCG GCC AAC AGC GAC GGC ACG GTG GCT TGC AAC ATG CTC ATG CCA GAG CCC GCT

            220                                     230
Gln Arg Trp Leu Val Gly Phe Val Leu Tyr Thr Phe Leu Met Gly Phe Leu Leu Pro Val
CAA CGC TGG CTG GTG GGC TTC GTG TTG TAC ACA TTT CTC ATG GGC TTC CTG CTG CCC GTG

            240                                     250
Gly Ala Ile Cys Leu Cys Tyr Val Leu Ile Ile Ala Lys Met Arg Met Val Ala Leu Lys
GGG GCT ATC TGC CTG TGC TAC GTG CTC ATC ATT GCT AAG ATG CGC ATG GTG GCC CTC AAG

            260                                     270
Ala Gly Trp Gln Gln Arg Lys Arg Ser Glu Arg Lys Ile Thr Leu Met Val Met Met Val
GCC GGC TGG CAG CAG CGC AAG CGC TCG GAG CGC AAG ATC ACC TTA ATG GTG ATG ATG GTG

            280                                     290
Val Met Val Phe Val Ile Cys Trp Met Pro Phe Tyr Val Val Gln Leu Val Asn Val Phe
GTG ATG GTG TTT GTC ATC TGC TGG ATG CCT TTC TAC GTG GTG CAG CTG GTT AAC GTG TTT

            300                                     310
Ala Glu Gln Asp Asp Ala Thr Val Ser Gln Leu Ser Val Ile Leu Gly Tyr Ala Asn Ser
GCT GAG CAG GAC GAC GCC ACG GTG AGT CAG CTG TCG GTC ATC CTC GGC TAT GCC AAC AGC

            320                                     330
Cys Ala Asn Pro Ile Leu Tyr Gly Phe Leu Ser Asp Asn Phe Lys Arg Ser Phe Gln Arg
TGC GCC AAC CCC ATC CTC TAT GGC TTT CTC TCA GAC AAC TTC AAG CGC TCT TTC CAA CGC

            340                                     350
Ile Leu Cys Leu Ser Trp Met Asp Asn Ala Ala Glu Glu Pro Val Asp Tyr Tyr Ala Thr
ATC CTA TGC CTC AGC TGG ATG GAC AAC GCC GCG GAG GAG CCG GTT GAC TAT TAC GCC ACC

            360                                     370
Ala Leu Lys Ser Arg Ala Tyr Ser Val Glu Asp Phe Gln Pro Glu Asn Leu Glu Ser Gly
GCG CTC AAG AGC CGT GCC TAC AGT GTG GAA GAC TTC CAA CCT GAG AAC CTG GAG TCC GGC

            380                 Ala             Ser 390 391
Gly Val Phe Arg Asn Gly Thr Cys Thr Ser Arg Ile Thr Thr Leu OPA
GGC GTC TTC CGT AAT GGC ACC TGC ACG TCC CGG ATC ACG ACG CTC TGA GCCCGGGCCACGCAG
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B

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                  1 Glu     Ser Ser     Gln             10      Gln Val     Val
                Met Asp Met Ala Asp Glu Pro Leu Asn Gly Ser His Thr Trp Leu Ser
ACTGAAAAGCAGCC  ATG GAC ATG GCG GAT GAG CCA CTC AAT GGA AGC CAC ACA TGG CTA TCC

Ser         20                  Leu Gly Pro Ser     Gly
Ile Pro Phe Asp Leu Asn Gly Ser Val Val Ser Thr Asn Thr Ser Asn Gln Thr Glu Pro
ATT CCA TTT GAC CTC AAT GGC TCT GTG GTG TCA ACC AAC ACC TCA AAC CAG ACA GAG CCG

            Met                                     50                  Val Val
Tyr Tyr Asp Leu Thr Ser Asn Ala Val Leu Thr Phe Ile Tyr Phe Val Val Cys Ile Ile
TAC TAT GAC CTG ACA AGC AAT GCA GTC CTC ACA TTC ATC TAT TTT GTG GTC TGC ATC ATT

            60                                      70
Gly Leu Cys Gly Asn Thr Leu Val Ile Tyr Val Ile Leu Arg Tyr Ala Lys Met Lys Thr
GGG TTG TGT GGC AAC ACA CTT GTC ATT TAT GTC ATC CTC CGC TAT GCC AAG ATG AAG ACC

            80                                      90
Ile Thr Asn Ile Tyr Ile Leu Asn Leu Ala Ile Ala Asp Glu Leu Phe Met Leu Gly Leu
ATC ACC AAC ATT TAC ATC CTC AAC CTG GCC ATC GCA GAT GAG CTC TTC ATG CTG GGT CTG

            100                                     110
Pro Phe Leu Ala Met Gln Val Ala Leu Val His Trp Pro Phe Gly Lys Ala Ile Cys Arg
CCT TTC TTG GCT ATG CAG GTG GCT CTG GTC CAC TGG CCC TTT GGC AAG GCC ATT TGC CGG

            120                                     130
Val Val Met Thr Val Asp Gly Ile Asn Gln Phe Thr Ser Ile Phe Cys Leu Thr Val Met
GTG GTC ATG ACT GTG GAT GGC ATC AAT CAG TTC ACC AGC ATC TTC TGC CTG ACA GTC ATG

            140                                     150
Ser Ile Asp Arg Tyr Leu Ala Val Val His Pro Ile Lys Ser Ala Lys Trp Arg Arg Pro
AGC ATC GAC CGA TAC CTG GCT GTG GTC CAC CCC ATC AAG TCG GCC AAG TGG AGG AGA CCC

            160         Asn Val             Cys     170
Arg Thr Ala Lys Met Ile Thr Met Ala Val Trp Gly Val Ser Leu Leu Val Ile Leu Pro
CGG ACG GCC AAG ATG ATC ACC ATG GCT GTG TGG GGA GTC TCT CTG CTG GTC ATC TTG CCC

            180                                     190
Ile Met Ile Tyr Ala Gly Leu Arg Ser Asn Gln Trp Gly Arg Ser Ser Cys Thr Ile Asn
ATC ATG ATA TAT GCT GGG CTC CGG AGC AAC CAG TGG GGG AGA AGC AGC TGC ACC ATC AAC

            200                                     210     Ala
Trp Pro Gly Glu Ser Gly Ala Trp Tyr Thr Gly Phe Ile Ile Tyr Thr Phe Ile Leu Gly
TGG CCA GGT GAA TCT GGG GCT TGG TAC ACA GGG TTC ATC ATC TAC ACT TTC ATT CTG GGG

            220                                     230
Phe Leu Val Pro Leu Thr Ile Ile Cys Leu Cys Tyr Leu Phe Ile Ile Ile Lys Val Lys
TTC CTG GTA CCC CTC ACC ATC ATC TGT CTT TGC TAC CTG TTC ATT ATC ATC AAG GTG AAG

            240                                     250
Ser Ser Gly Ile Arg Val Gly Ser Ser Lys Arg Lys Lys Ser Glu Lys Lys Val Thr Arg
TCC TCT GGA ATC CGA GTG GGC TCC TCT AAG AGG AAG AAG TCT GAG AAG AAG GTC ACC CGA

            260                                     270
Met Val Ser Ile Val Val Ala Val Phe Ile Phe Cys Trp Leu Pro Phe Tyr Ile Phe Asn
ATG GTG TCC ATC GTG GTG GCT GTC TTC ATC TTC TGC TGG CTT CCC TTC TAC ATA TTC AAC

            280     Val                             290
Val Ser Ser Val Ser Met Ala Ile Ser Pro Thr Pro Ala Leu Lys Gly Met Phe Asp Phe
GTT TCT TCC GTC TCC ATG GCC ATC AGC CCC ACC CCA GCC CTT AAA GGC ATG TTT GAC TTT

        Ile 300                                     310
Val Val Val Leu Thr Tyr Ala Asn Ser Cys Ala Asn Pro Ile Leu Tyr Ala Phe Leu Ser
GTG GTG GTC CTC ACC TAT GCT AAC AGC TGT GCC AAC CCT ATC CTA TAT GCC TTC TTG TCT

            320                                     330                     Glu
Asp Asn Phe Lys Lys Ser Phe Gln Asn Val Leu Cys Leu Val Lys Val Ser Gly Thr Asp
GAC AAC TTC AAG AAG AGC TTC CAG AAT GTC CTC TGC TTG GTC AAG GTG AGC GGC ACA GAT

            340                                     350
Asp Gly Glu Arg Ser Asp Ser Lys Gln Asp Lys Ser Arg Leu Asn Glu Thr Thr Glu Thr
GAT GGG GAG CGG AGT GAC AGT AAG CAG GAC AAA TCC CGG CTG AAT GAG ACC ACG GAG ACC

            360                                 369
Gln Arg Thr Leu Leu Asn Gly Asp Leu Gln Thr Ser Ile OPA
CAG AGG ACC CTC CTC AAT GGA GAC CTC CAA ACC AGT ATC TGA ACTGCTTGGGGGTGGAAAGAA
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FIG. 1. Primary structure of two SSTRs. Nucleotide and predicted amino acid sequences of the human SSTR1 (hGPR81) (*A*) and SSTR2 (*B*) genomic clones. Amino acid residues of the corresponding mouse proteins that differ from those of the human SSTRs are shown above the human sequences.

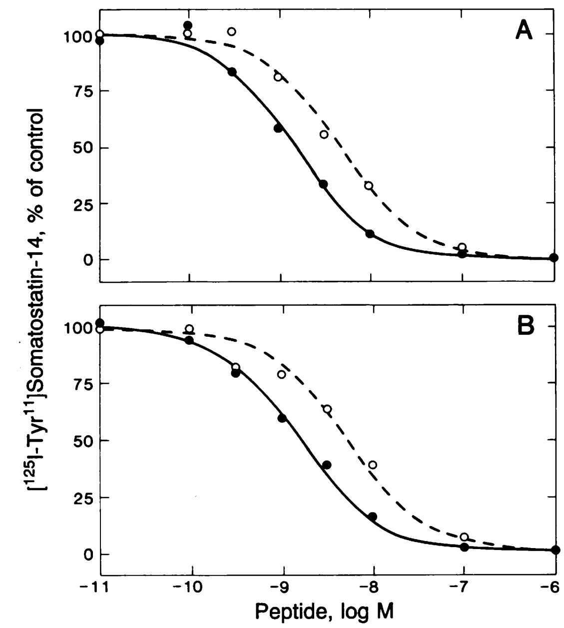

FIG. 3. Binding of [^{125}I-Tyr^{11}]somatostatin-14 to CHO cells expressing SSTR1 and SSTR2. (*A*) Cells transfected with human SSTR1. (*B*) Cells transfected with mouse SSTR2. Data (the mean of duplicate determinations) have been normalized to account for radiolabeled ligand bound in the absence of inhibitor (control binding, 1425 cpm for *A* and 2080 cpm for *B*) and have been corrected for nonspecific binding (radiolabeled ligand bound in the presence of 1 μM somatostatin-14, 225 cpm in both cases). Inhibition of binding in the presence of somatostatin-14 (●) and somatostatin-28 (○) is shown. Nontransfected CHO cells bound 200 and 156 cpm of radiolabeled ligand in the absence and presence of 1 μM somatostatin-14, respectively. Curves have been modeled in terms of ligand interaction with a single population of binding sites.

were amplified by using PCR as described by Libert *et al.* (11). The PCR products were cloned and sequenced, and clones encoding two new putative receptors were obtained, termed hGPR81 and hGPRi5. Of 24 different clones that were sequenced, 19 encoded hGPR81 and 3 encoded hGPRi5; the sequences of the remaining 2 clones were unrelated to those of members of the G protein-coupled receptor superfamily (17). As the genes encoding G protein-coupled receptors often lack introns (18), the genes encoding these two new receptors were isolated and sequenced. The sequence of the genomic fragment encoding hGPR81 revealed a 1173-bp open reading frame (Fig. 1*A*) encoding a 391-amino-acid protein (M_r = 42,657). This protein has seven putative transmembrane domains and the extracellular NH_2-terminal segment preceding the first membrane-spanning segment has three consensus sites for N-linked glycosylation (Asn-4, Asn-44, and Asn-48). There are two putative phosphorylation sites for cAMP-dependent protein kinase (19) (Thr-172 and Ser-265) in regions that are predicted to be intracellular. The intracellular COOH-terminal domain is also serine- and threonine-rich and could serve as a substrate for serine/threonine protein kinases (20). In addition, several amino acids that are conserved within the superfamily of G protein-coupled receptors (17, 21) are also conserved in hGPR81 (Fig. 2). To confirm that hGPR81 was encoded by a gene that lacked introns, the gene for the mouse homolog was isolated and sequenced. There is 99% identity between the amino acid sequences of the human and mouse proteins (Fig. 1*A*). This high degree of sequence identity suggests that all domains of this protein are functionally important.

Functional Properties of Human SSTR1. Since the presence of a short cytoplasmic loop connecting transmembrane segments M5 and M6 is a feature of G protein-coupled receptors that bind peptide hormones and neuropeptides (22), peptides known to regulate hormone secretion from pancreatic islets were tested as ligands for hGPR81. Attempts to identify the ligand for hGPR81 by injecting RNA transcribed *in vitro* into *Xenopus laevis* oocytes (23) and assessing changes in basal or stimulated cAMP levels in response to various peptides [calcitonin-gene-related peptide, gastric inhibitory polypeptide, glucagon, glucagon-like peptide 1-(7–36 amide), and vasoactive intestinal peptide], or Ca^{2+} efflux (24) (angiotensin II, bombesin, cholecystokinin, and vasopressin) were unsuccessful. We next examined the binding of radiolabeled peptides (galanin, glucagon, and somatostatin) to COS-7 cells transiently expressing hGPR81. When transfected cells were incubated with [^{125}I-Tyr^{11}]somatostatin-14 and the plates were subjected to autoradiography, positive signals were detected that could be competitively blocked by addition of excess unlabeled somatostatin-14, suggesting that hGPR81 was a SSTR, designated SSTR1. The pharmacological characteristics of SSTR1 were examined in CHO cells that were stably expressing this receptor. These cells exhibited specific binding of [^{125}I-Tyr^{11}]somatostatin-14 (Fig. 3*A*) with a higher affinity for somatostatin-14 than somatostatin-28, having inhibitory concentration for half-maximal response (IC_{50}) values of 1.5 and 4.7 nM, respectively. These values are

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hSSTR1    1 MFPNGTASSPSSSPSPSPGSCGEGGGSRGPGAGAADGMEEPGRNASQNGTLSEGQGSAILISFIYSVVCLVGLCGNSMVI
            *     *  *            |*| |  | |   |*   ||  | *  |  |  | *|* |*** ***||*****||**
hSSTR2    1 M:::DMADEPL:::::::::::NGSHTWLSIPFDLNGSVVSTNTSNQTEPYYDLTSNAVL:TFIYFVVCIIGLCGNTLVI
                                                                     <------------M1--------

hSSTR1   81 YVILRYAKMKTATNIYILNLAIADELLMLSVPFLVTSTLLRHWPFGALLCRLVLSVDAVNMFTSIYCLTVLSVDRYVAVV
            *********** ************** **||***     * *****  |**|*||**||* ****|****|*|***|***
hSSTR2   66 YVILRYAKMKTITNIYILNLAIADELFMLGLPFLAMQVALVHWPFGKAICRVVMTVDGINQFTSIFCLTVMSIDRYLAVV
            --->          <-----------M2----------->           <---------M3--------->

hSSTR1  161 HPIKAARYRRPTVAKVVNLGVWVLSLLVILPIVVFSRTAANSDGTVACNMLMPEPAQRWLVGFVLYTFLMGFLLPVGAIC
            ****|*||***  **|| ||** |********||||   |*  *  |* |  *  |  *  **||***||***|*|| **
hSSTR2  146 HPIKSAKWRRPRTAKMITMAVWGVSLLVILPIMIYAGLRSNQWGRSSCTINWPGESGAWYTGFIIYTFILGFLVPLTIIC
                        <----------M4---------->                       <-------------M5-----

hSSTR1  241 LCYVLIIAKMRMVALKAGWQQRKRSERKITLMVMMVVMVFVICWMPFYVVQL::::VNVFAEQDDATVSQLSVILGYANS
            ***| ** *||  ||| *   **|**|*|* ** |** **| **|***| ||    | | |     || |  *|*|****
hSSTR2  226 LCYLFIIIKVKSSGIRVGSSKRKKSEKKVTRMVSIVVAVFIFCWLPFYIFNVSSVSMAISPTPALKGMFDFVVVLTYANS
            --------->                  <--------------M6-------------->      <---------M7--

hSSTR1  317 CANPILYGFLSDNFKRSFQRILCLSWMDNAAEEPVDYYATALKSRAYSVEDFQPENLESGGVFRNGTCTSRITTL  391
            *******|*******|*** |***  |  |||||    |   ***     | *   *||             *||
hSSTR2  306 CANPILYAFLSDNFKKSFQNVLCLVKV:SGTDDGERSDSKQDKSRLNETTETQRTLLNGD::::::::::LQTSI  369
            ---------->
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FIG. 2. Comparison of the amino acid sequences (in single-letter code) of human SSTR1 and SSTR2. Asterisks denote identical amino acids, and bars indicate similar residues. Gaps introduced to generate this alignment are represented by colons. The seven predicted transmembrane domains (M1–M7) are noted. The amino acid residues that are conserved within the superfamily of G protein-coupled receptors are shown in boldface type.

similar to those reported previously for the affinity of somatostatin-14 and -28 for their receptors (9, 10, 25).

Cloning and Functional Expression of SSTR2. Hybridization of a human SSTR1 probe to a Southern blot of *Eco*RI-digested human DNA showed a strong signal at 14 kilobases (kb), the size expected for the *Eco*RI fragment containing this gene, as well as weaker hybridization to fragments of >23, 18, 8.4, and 7.8 kb (Fig. 4). This probe also hybridized to multiple fragments in an *Eco*RI digest of mouse DNA of >23, 13.5, 8.6, and 8.2 kb. The presence of hybridizing fragments, in addition to those expected, suggested that there was a family of SSTRs as noted previously for the adrenergic and dopaminergic receptors (18, 26).

By screening human and mouse genomic libraries with a human SSTR1 probe, we isolated the gene encoding a second putative SSTR, termed SSTR2; the *Eco*RI fragments containing the human and mouse SSTR2 genes are 18 and 8.2 kb, respectively and are noted in Fig. 4. The nucleotide sequence of the human SSTR2 gene revealed a 1107-bp open reading frame encoding a 369-amino acid protein (M_r = 41,305) (Fig. 1*B*) that has seven transmembrane segments, four putative N-glycosylation sites (Asn-9, Asn-22, Asn-29, and Asn-32) in the extracellular NH_2-terminal segment, and one site that could be phosphorylated by cAMP-dependent protein kinase (18) (Ser-250). There is 94% identity between the amino acid sequences of human and mouse SSTR2 (Fig. 1*B*).

Comparison of the amino acid sequences of human SSTR1 and SSTR2 indicates that there is 46% identity and 70% similarity between these two receptors (Fig. 2). The regions of greatest identity are the transmembrane domains, especially M1, M2, and M7, implying that perhaps these regions may be involved in ligand binding. The pharmacological properties of SSTR2 are similar to those of SSTR1, with a higher affinity for somatostatin-14 than -28. The IC_{50} values are 1.6 and 5.2 nM, respectively (Fig. 3*B*).

Tissue Distribution of SSTR1 and SSTR2 mRNAs. The tissue distribution of SSTR1 and SSTR2 was examined by RNA blotting. Human SSTR1 mRNA was 4.8 kb in size and was expressed at highest levels in jejunum and stomach (Fig. 5*A*); it also was readily detected in human islet RNA (data not shown). There were low levels of SSTR1 mRNA in colon, colon carcinoma, and kidney. Although SSTR1 mRNA could not be detected in Northern blots of human cerebrum RNA prepared from a part of the temporal lobe, it could be seen readily in blots of RNA prepared from the entire mouse brain, indicating that this receptor subtype was expressed in brain (data not shown). The tissue distribution of SSTR2 was quite different from that of SSTR1. Human SSTR2 mRNA was

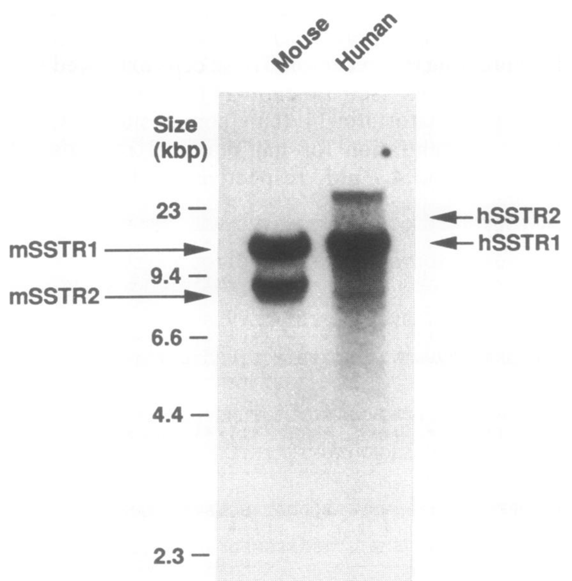

FIG. 4. Southern blot analysis of human and mouse genomic DNA. Ten micrograms of human and 10 μg of mouse genomic DNA were digested with *Eco*RI, electrophoresed in a 1% agarose gel, blotted onto a nitrocellulose filter, and hybridized with a ^{32}P-labeled human SSTR1 probe as described (13). The *Eco*RI fragments containing human (h) and mouse (m) SSTR1 and SSTR2 genes are indicated by arrows.

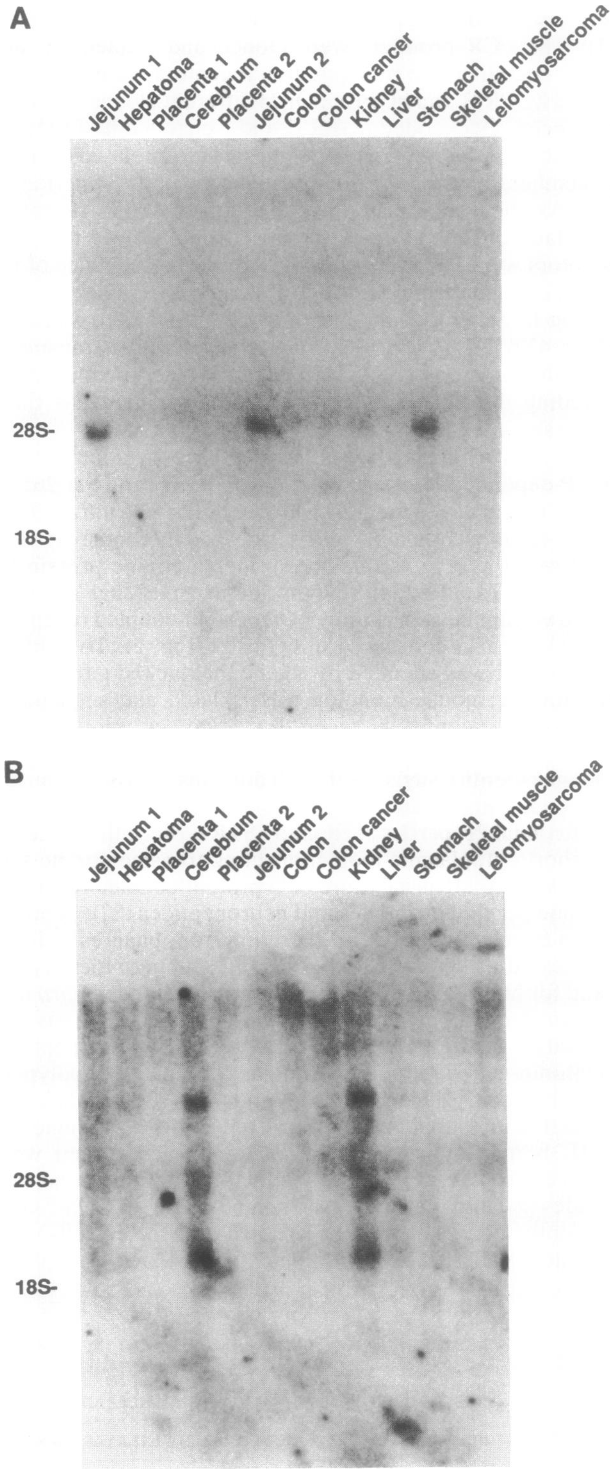

FIG. 5. Northern blot analyses of SSTR1 and SSTR2 mRNA in human tissues. Twenty micrograms of total RNA was denatured with glyoxal, electrophoresed in a 1% agarose gel, blotted onto a nylon membrane, and hybridized with ^{32}P-labeled human SSTR1 (*A*) or human SSTR2 (*B*) probes.

expressed at highest levels in cerebrum and kidney and in both tissues, two transcripts of 8.5 and 2.5 kb hybridized with the SSTR2 probe (Fig. 5B). Low levels of SSTR2 mRNA were detected in jejunum, hepatoma, colon, colon carcinoma, and liver. SSTR2 mRNA was also present in rat pancreatic islets (data not shown), implying that pancreatic islets express both SSTR1 and SSTR2.

The present results indicate that the biological effects of somatostatin are mediated by tissue-specific expression of a family of SSTRs. Cloning and Southern blotting studies suggest that this gene family may comprise perhaps four members. The sequences and ligand-binding properties of two of these receptors, SSTR1 and SSTR2, have been determined. Although both possess a similar affinity for somatostatin-14 and -28, their sequences are surprisingly divergent. This sequence divergence may well underlie the diverse biological effects of somatostatin (2) by coupling its receptors to different intracellular effector systems (27, 28). The cloning of SSTR1 and SSTR2 should lead to a better understanding of the molecular basis for SSTR heterogeneity as determined by cross-linking analyses (29–32) and the mechanisms and regulation of somatostatin function. In addition, they should facilitate the development of selective analogues for specific diagnostic and therapeutic applications (33, 34).

We thank Drs. D. W. Scharp and P. E. Lacy, Islet Transplantation Laboratory, Washington University School of Medicine, St. Louis, for providing human islets; Dr. C. Logsdon, University of Michigan, Ann Arbor, for carrying out the Ca^{2+} efflux assays; Dr. L. Chasin, Columbia University, New York, for the CHO cell line, DG44; and Dr. H. Fukumoto, Kyoto University School of Medicine, Mr. P. Rubinstein, and Ms. M. Seino for their expert technical assistance. This work was supported by the Howard Hughes Medical Institute, National Institutes of Health grants (DK-18347, DK-20595, and DK-42086), and Novo Nordisk (Japan). S.R.P. is the recipient of traineeships from National Institutes of Health (GM-07183 and GM-08369).

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